

Molecular identification of Glucose Transporter 4 and MyoD of *Therapon jarbua* Skeletal Muscle from Polluted Ennore Creek

Suresh M¹, Ameer Kalander ²and Mazher Sultana³

¹ & ³ Unit of Human Health and Environmental Biotechnology, Department of Advanced Zoology and Biotechnology, Faculty of Science, Presidency College, Chennai 600005, Tamilnadu, INDIA

² Associate Professor in Clinical Biochemistry, College of Medical Sciences, Haramya University, Harar, ETHIOPIA
sultanaafzal@yahoo.com

ABSTRACT

One of the major public health concerns in recent years has been the Environmental Pollution and its effects on the living organism. Population explosion, urbanization, industrialization and human apathy have all contributed towards increasing quantities of pollutants leading to an “ecological disaster”. Of late, the coastal ecosystems are highly degraded due to high population and industrial growth. The increased accumulation of anthropogenic trace and toxic metals in the north Chennai harbour area, leads to high toxicity and tendency to bioaccumulation. At present, the coastal pollution endangers Ennore Creek by industrial, municipal, domestic sewage disposal and run off during rainy season. A fish *Therapon jarbua* was collected directly from polluted sites of Ennore Creek to analyze the Myo D and Glut 4 protein (Glucose Transporter Type 4) in the skeletal muscle. This study showed significant variation ($p < 0.05$) in the expression of Myo D and Glut 4 protein under the pollutant impact. These molecular changes are expected due to the influence of various pollutants in the Ennore Creek water.

Key words: Environmental Pollution, Ennore Creek, *Therapon jarbua*, Myo D and Glut4 protein.

INTRODUCTION

Our environment consists of air, water and soil. The air we breathe the water we drink and the soil we use to produce food may be polluted by undesired or toxic substances. Since the industrial revolution, industries have been booming and, consequently, millions of anthropogenic compounds have entered our environment. Persistent organic pollutants have been found even in remote areas of the world [1]. Pollution may be defined as the presence of undesired natural or anthropogenic substances in our environment or a chemical that exceeds normal background level and has the potential to cause harm. Harm includes biochemical or physiological changes that adversely affect an individual, organism's ability to breed, grow or survive [2]. One of the major public health concerns in recent years has been the Environmental Pollution and its effects on the living organism. Population explosion, urbanization, industrialization and human apathy have all contributed towards increasing quantities of pollutants leading to an “ecological disaster”. Of late, the coastal ecosystems are highly degraded due to high population and industrial growth [3, 4]. Due to various pollution including pesticide poisoning [5] also. Over exploitation of water resources by power plant industries and the municipal uses and encroachment for urban development's force the fishing community to the brink of disappearance. When those natural resources are imperiled, so too are the livelihoods of the many people who live and work there. The environmental abuse and the negligence of the governing body make the matter worse [6]. The severity of water pollution of Ennore Creek and its coastal areas makes the ecosystem unsustainable in which some rare fishes and plants ultimately thrive [7]. The affected fishing communities start migrating to other places for want of a suitable environment in order to improve their economic status. Together they deteriorate the skills for the optimal utilization of

coastal resources in tune with the nature. Thus, the guards of coastal ecosystem tend to disappear from the scene of sustainable marine resource utilization only leaving the fragile resources at the mercy of profit centered industries with environmental ignorance. The increased accumulation of anthropogenic trace/toxic metals in the north Chennai harbour, Cuvum and Adyar marine environments is less desirable by-products of industrialized society of these regions because of their extreme persistence, high toxicity and tendency to bioaccumulation [8, 9]. The study of Palanisamy Shanmugam *et al.*, [10] revealed the variations in metal concentrations before and during monsoonal storms. It is observed that copper concentrations during the monsoonal storm are found to be higher than the allowable limit (0.02 mg/l). The abrupt increase in copper concentrations is due to surface runoff and contributions of river and pipeline discharges to the coastal system. In both periods, the manganese concentrations are within the permissible limit (0.1 ppm) in all sample locations, though increased levels of magnesium at Annai Sivagami Nagar can be attributed to the concentrated municipal wastes accumulated before storms prevailed. It is evident from their results that the concentrations of Nickel, Cobalt, Lead and Cadmium appeared to be very high during September and exceeded the maximum permissible limit (0.01, 0.005, 0.1 and 0.01 respectively) in most of the sample locations. As a result of monsoonal storms during October, these concentrations were considerably decreased to be within the permissible limit of the international standards. The creeks are comparatively less along Tamil Nadu coast. The study of Ennore and Kovalam creeks are also dynamically changing due to seasonal variation [11,12]. Hence the present investigation was carried out to evaluate the Ennore Creek water by selecting a fish, *Therapon jarbua*. MyoD and Glut 4 was analysed as biomarkers of aquatic pollution in the Ennore Creek.

LOCATION OF STUDY AREA:

The area of the study is the Ennore creek which is situated at 13°14' 051''N latitude and 80°19'911'' E longitude and close to the northern boundary of Chennai City Ennore Creek traditionally influences the livelihood of the stakeholders inhabited near the creek. The Thalamkuppam area is located 13°13'323'' N latitude and 80°19'704'' E longitude. Ennore Creek is located in Thiruvallur district of Tamil Nadu. Ennore creek was once the paradise for mangrooves, reptiles, turtles and rare fishes. The study area is not only the nature's gift but also a source for sustaining the traditional fishermen community settled in this Creek [13,14]. The Creek, situated in between the Kourtaliar river (freshwater source) and the Bay of Bengal is intercepted by Buckingham canal (tidal water body) and has been supporting the livelihood of many thousands of fishing families who are the original stakeholders settled in the nearby villages. This estuary formed a good source of fisheries, particularly of mullets and prawns. Studies on the hydrobiology and fisheries of this water area are therefore being pursued regularly at the fisheries biological station at Ennore. At present, the coastal pollution endangers Ennore Creek by all means.

MATERIALS AND METHODS

The fish *Therapon jarbua* was collected directly from polluted sites of Ennore Creek. The skeletal muscle was analysed for Myo D and Glut 4 protein (Glucose Transporter Type 4) by the method of Laemmle, 1970 [15].

QUANTIFICATION OF PROTEIN USING NANO DROP

The amount of protein is quantified using Nano drop instrument and with the help of a software. The protein is estimated in µg/µl. While loading the samples in the gel, the amount of protein and the sample loading buffer must be equal. Hence the amount of protein to be added can be calculated accordingly.

RESULTS

Biomarkers of protein expression, Myo D and Glut 4 were shown associated with aquatic pollutants. While this was studied largely in muscles, there is hardly any data whether this phenomenon reflects in target tissues affected in pollutants. Therefore, the aim of this study is to detect Myo D and Glut 4 protein from the brackish water fish *Therapon jarbua*, collected from Ennore Creek and to test whether these muscle exhibit protein markers. Various pollutants, fish protein expression was confirmed by physico-chemical study [16] (Table – 1), Western blot, cells under polluted water conditions exhibited increased protein expression of Myo D and Glut 4 (Fig- 1,2). Despite the fact that pollution was induced by various sources, this study showed significant variation ($p < 0.05$) (Table: 2 and 3) in the expression of Myo D and Glut 4 protein under the impact of pollutants. These molecular changes are expected due to the influence of various pollutants in the Ennore Creek water.

Table 1. PHYSICO CHEMICAL PARAMETERS OF POLLUTED SITES OF ENNORE CREEK AND THALAMKUPPAM.

S.No	Parameters	Standard (ISI)	Polluted site 1 Ennore Creek	Polluted site 2 Thalamkuppam
1	pH	7.8 – 8.3	7.2	7.6
2	Temperature 0C	30	27.5	28.5
3	Odour	Unobjectionable	Objectionable	Objectionable
4	Turbidity (NTU)	10 NTU or less	13.2	14.5
5	TDS		38950	33280
6	TSS	25mg L-1 or less	795	760
7	Dissolved Oxygen	4mg L-1 or less	6.3	6.5
8	BOD Biochemical Oxygen Demand	30mg L-1 or less	32	38.65
9	COD Chemical Oxygen Demand	250mg L-1 or less	786	925
10	Nitrate	10mg L-1 or less	0.862	0.873
11	Nitrite	10mg L-1 or less	76.43	80.5
12	Phosphorus as Phosphate PO4-3	15mg L-1 or less	1.35	1.42
Table1 b.Trace / Toxic metals of Polluted Sites of Ennore creek and Thalamkuppam				
1	Cadmium	0.01 mg L-1	0.07	0.09
2	Lead	0.1 mg L-1	2.13	2.84
3	Copper	0.02 mg L-1	2.7	3.5
4	Nickel	0.01 mg L-1	1.52	1.98
5	Zinc	0.1 mg L-1	1.9	2.52
6	Iron	0.1 mg L-1	3.16	3.94
7	Manganese	0.1 mg L-1	0.73	1.05
8	Chromium	0.1 mg L-1	1.8	3.03
9	Cobalt	0.005 mg L-1	0.96	1.68

Table 2: Myo D Protein expression of skeletal muscle of *Therapon jarbua*.

MyoD	1	2	3	Mean	act	g/actin	SD
control	576.2	576	576.02	576.0733	2277.991	0.252887	0.110151
p 1	2134.355	2134	2134.01	2134.122	2239.87	0.952788	0.202134
p 2	2730.6	2730	2730.04	2730.213	2293.506	1.19041	0.33546

Table 3: Glu4 Protein expression of skeletal muscle of *Therapon jarbua*

Glut4	1	2	3	Mean	act	g/actin	SD
control	3061.02	3061	3061	3061.007	2277.991	1.343731	0.011547
p 1	2009.01	2009.5	2009	2009.17	2239.87	0.897003	0.285832
p 2	838.284	838	838	838.0947	2293.506	0.365421	0.163967

Fig: 1. Myo D Protein expression of skeletal muscle of *Therapon jarbua*.

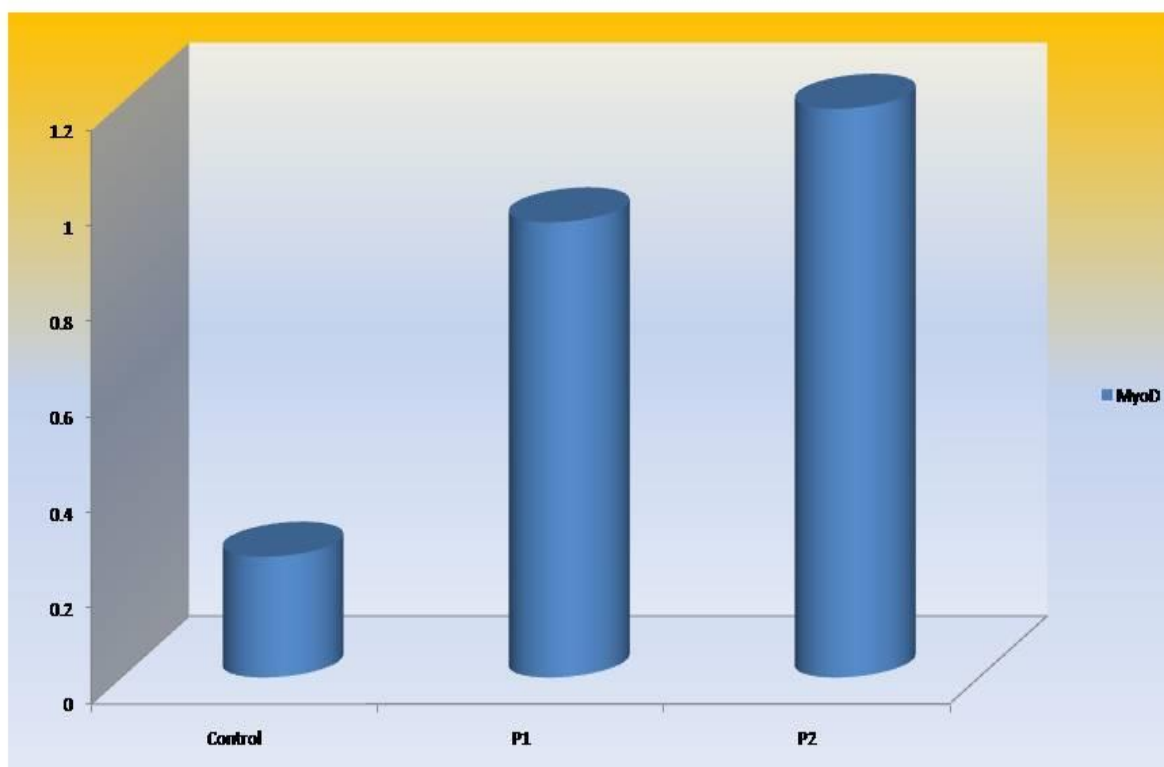
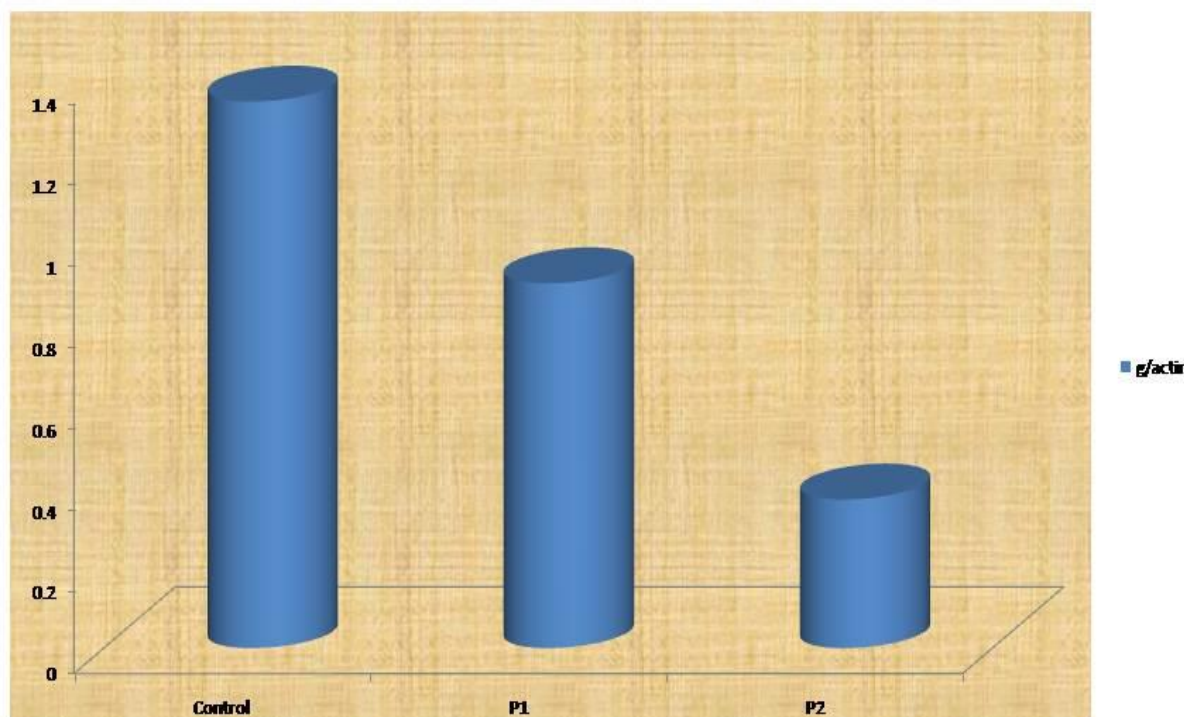


Fig 2: Glu4 Protein expression of skeletal muscle of *Therapon jarbua*



REFERENCES

- [1] Ballschmiter, K., Hackenberg, R., Jarman, W.M. and Looser, R. (2002) Man-made Chemicals found in remote areas of the world. The experimental definition for POPs. Environ. Sci. & Pollut. Res., 9 (4):274-288.
- [2] Walker C.H., Hopkins S.P., Sibly, R.M., and Peakall D.B. (1996) Principles of Ecotoxicology. London. Yaylor and Francis. 321.
- [3] Glasby Y., and Roonwal G.S. (1995) Marine Population in India: An emerging problem. Curr. Sci. 68, 495-497.
- [4] UNEP. (1997) Global Environment Outlook 1. Global State of the Environment Report. 8-12.
- [5] Sen Gupta. (1990) State of the marine environment in the South Asian Seas Region. UNEP, Regional Seas Reports and Studies. No: 123. 19.
- [6] Sreenivasan, R., and Franklin, T. (1975) Effects of disposal of effluents from petrochemical complex on Ennore Backwaters. Bulletin of the Department of Marine Science, University of Cochin, 7, 273-280.
- [7] NEERI. (1995) Coastal aquaculture, Report. 7.
- [8] De Santo R.S. (1991) Inorganic contaminants of surface water. Springer. Verlag Pub. New York.
- [9] Clark, R.B. (1992) Marine pollution, 3rd ed. Clarendon Press, Oxford, U.K.
- [10] Palanisamy Shanmugam, Neelamani, S; Yu Hwun Ahn., Ligy Philip, Gi-Hoon Hong. (2006) Assessment of Levels of Coastal marine pollution of Chennai city, Souhtern India, Water Resource Management, 217, 1187-1206.
- [11] Durariraj, S. (1998) Application of remote data for coastal zone mapping – A case study of the part of east coast of Tamil Nadu. M.Tech. Thesis, Anna University, Madras.138.
- [12] Manivel, M., Manikiam, B., Kumaran Raju, D., and Mosses Edwin. J. (1995) Study of ecosystem development along Coramandal coast of Tamil Nadu using remote sensing techniques. GISdevelopment.net. AARS, Poster Session 3.
- [13] Jayaprakash, M. (2003) Geochemical Assessment of Heavy Metal Pollution in Ennore Creek. North of Chennai. Ph.D.Thesis, Madras University, Chennai.
- [14] Jayaprakash, M., Srinivasalu S., Jonathan M.P., and Mohan V. (2005) A baseline study of physico-chemical parameters and trace metals in water of

- Ennore Creek, Chennai, India. Marine Pollution Bullett. 50 (5), 583-589.
- [15] Laemmli U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680 - 685.
 - [16] APHA (1998) Standard methods for examination of water and waste water. American Public Health Organisation, Washington DC, USA.
 - [17] Arunagiri et al., (1992) Modelling of pollution level in Ennore Creek. Project Report. Anna University, Madras.
 - [18] SPIC-SMO. (1990) Republic of India, Government of Tamil Nadu Report.3-17.
 - [19] Ganesan, S., and Mazher Sultana, 2012. Molecular and Biochemical Evidences for Ecotoxicological impact on freshwater fish from Chromepet Lake, Chennai. Bharathiyar University, Coimbatore, Tamilnadu, India.
 - [20] Kahn, B.B., and Lilly, L. (1996) Glucose transport: pivotal step in insulin action. Diabetes 45: 1644-1654.
 - [21] Planas, J.V, Capilla, E., and Gutierrez, J. (2000) Molecular identification of a glucose transporter from fish muscle. FEBS Lett 481: 266-270.